Variations on a theme: Changes in the floral ABCs in angiosperms

Anneke S. Rijpkema, Michiel Vandenbussche, Ronald Koes, Klaas Heijmans, Tom Gerats

Angiosperms display a huge variety of floral forms. The development of the ABC-model for floral organ identity, almost 20 years ago, has created an excellent basis for comparative floral development (evo-devo) studies. These have resulted in an increasingly more detailed understanding of the molecular control circuitry of flower development, and the variations in this circuitry between species with different types of flowers. In this review, we analyze the variations in the molecular control of floral organ development: the changes in the floral ABCs. In addition, we discuss the control and diversification of inflorescence architecture, as this is another important source of structural diversity between flowering species.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

One of the most important contributions to our understanding of flower development was the formulation of the classic ABC-model for floral organ identity, by now almost 20 years ago. This model was based on an interpretation of Arabidopsis and Antirrhinum mutants [1], although in the first version of the Antirrhinum model no A-function was included [2]. In the early nineties, genes encoding B- and C-functions were cloned from both species, all of them members of the MADS-box gene transcription factor family [3–8]. A-function representatives were only cloned from Arabidopsis, and appeared to be an AP2 transcription factor gene [9] and the MADS-box gene AP1 [10].

The striking overall similarities in B- and C-function regulation between Arabidopsis and Antirrhinum, while fairly distantly related within the core eudicots, led to the assumption of a universal applicability of the ABC-model, although this viewpoint was not necessarily shared by the original authors and other researchers. Nevertheless, the development of the ABC-model was a major breakthrough in the understanding of floral development and has acted as a catalyst for comparative floral development studies (floral evo-devo). These have resulted in a presently much better understanding of the variation in the molecular control of the development of different types of flowers.

In general, we can distinguish two types of variability:
(1) Variations in the molecular networks controlling flower development, between species with similar flower architecture. A large proportion of this variability can be explained by lineage-specific differences in gene duplications and the subsequent functional diversification, leading to variations in functional redundancy, gene loss, and subfunctionalization. Moreover, there are indications that some aspects of the regulatory network are not conserved between certain species, although the final result, a four-whorled flower with sepals, petals, stamens and carpels, is the same. This demonstrates the plasticity of (molecular) evolution to generate different mechanisms to control the same process.

(2) Variations between species with flowers with different architectures. Given the enormous variation in floral forms among angiosperms, it is obvious that important ABC regulatory changes can be expected in flower types that deviate from the general sepal–petal–stamen–carpel set-up. Classic examples can be found in the monocots, such as the tulip flower, and the flowers of grasses which develop palea/lemma and lodicules rather than sepals and petals.

This chapter is complemented with a section on diversity of inflorescence architecture, which forms another important source of variation between flowering species.

2. Variations in the control of floral organ development

2.1. The A-function

In Arabidopsis the A-function has been attributed to two genes: MADS-box gene APETALA1 (AP1) and AP2/ERF transcription factor APETALA2 (AP2) [9,10]. In mutants of these genes, the sepals are transformed into leaf- or bract-like organs (or develop carpelloid features), and the petals are either absent or transformed into stamen-like structures. These genes thus appear to be required for the correct specification of the identity of sepals and petals. It is debatable whether AP1 and AP2 truly function as parianth organ identity genes (reviewed in [11]), they more likely “specify” organ identity indirectly by establishing the floral meristem [12] and by restricting C-function gene expression to the inner floral whorls [13,14]. Functional studies on AP1 lineage genes from other species indicate that the role of AP1 in floral meristem specification is most likely conserved in other eudicot species, while the contribution of the gene to perianth formation is not (reviewed in [11]).

In Arabidopsis, the crucial role of the AP2 gene in suppressing AGAMOUS (AG) in the perianth, in turn is regulated by the microRNA miR172 [15]. Recently, doubt has arisen about the universality of the role of AP2 genes in C-function gene regulation (e.g. [11]). Mutants of Petunia and Antirrhinum AP2 orthologs did not exhibit the same phenotype as Arabidopsis ap2 mutants [16,17]. The blind (bl) mutant in Petunia and fislata (fis) mutant in Antirrhinum, however, display a partial A-function phenotype, producing flowers with petals converted to antheroids [18–20].

Cartolano et al. [21] demonstrated that BL and FIS encode a homologous microRNA from the mir172/16 family. BL (Petunia) and FIS (Antirrhinum) are required to confine C-gene expression to the inner two floral whorls. Suppression is indirect, since C-function MADS-box genes do not harbor a mir169 target site sequence and thus cannot be direct targets. mir169 microRNAs are thought to target mRNAs of the NF-YA transcription factor family [22]. As NF-Y transcription factor complexes can activate target genes via CCAAT-boxes, which are present in the introns of C-function genes, Cartolano et al. [21] proposed that NF-YA members might be able to upregulate C-function gene expression. In this way, mirBL and mirFIS would repress expression of C-function genes by post-transcriptional repression of NF-YA members, although evidence for this is still lacking.

Two completely different mechanisms thus appear to have evolved to serve the same function: restricting C-function gene activity to the inner two floral whorls. This is clearly an example of variation in molecular networks without a structural difference in flower make-up. Remarkably, the elements of the miR169-NF-YA machinery are also present in Arabidopsis, while the AP2-miR172 elements can be found in Antirrhinum and Petunia. Future research will show whether these complementary mechanisms have lost some or all function, and/or acquired new ones. Moreover, it is important to examine which of the two mechanisms (or indeed yet other mechanisms) of restricting C-function gene activity to the center of the flower are employed by other angiosperm species.

This information can thus help us to unravel the evolutionary history, and level of conservation, of the miRNA169 and miRNA172 pathways.

2.2. The B-function

Arabidopsis and Antirrhinum both contain two B-function genes (APETALA3, AP3 plus PISTILLATA, PI; and DEFICIENS, DEF plus GLOBOSA, GLO, respectively), which are required to specify petal and stamen identity in the second and third floral whorls. All of their single mutants display the same homeotic transformation of petals to sepals and stamens to carpels. This is in accordance with the activity of the encoded proteins. DEF and GLO in Antirrhinum and AP3 and PI in Arabidopsis, as obligate heterodimers [3,4]. The expression of either of the B-function genes is initiated independently in the second and third floral whorls, but the maintenance of high levels of DEF and GLO or AP3 and PI by autoregulation depends upon the presence of the heterodimeric protein complex (Fig. 1) [23–26].

While the DEF/AP3 and GLO/PI lineages originated from a gene duplication that happened an estimated 260–290 MYA [27,28], it has become clear that in many species the B-function has been further shaped and complicated by other rounds of gene duplications in both gene lineages. Of special interest for the evolution of the core eudicot flower, is a duplication in the DEF/AP3 lineage which coincided with the radiation of the core eudicots, and resulted in the euAP3 lineage (to which DEF and AP3 belong) and the TM6 lineage [29]. euAP3 and TM6 proteins can easily be distinguished by their distinct C-terminal motifs, the so-called euAP3 and paleoAP3 motifs. Proteins containing a paleoAP3 motif can be found throughout the angiosperms, while euAP3 motif containing proteins are found only in the core eudicots. Remarkably, the euAP3 C-terminal motif seems to have originated from the paleoAP3 motif by a frameshift mutation [30,31]. Many core eudicots have retained both euAP3 and TM6 gene copies, while Arabidopsis and Antirrhinum both have lost the TM6 gene [29,32]. As a consequence, the function and regulation of TM6 genes was not included in the original ABC-model.

An early indication that B-function gene regulation might deviate from the original ABC-model in some eudicot species, despite having a similar floral architecture as Arabidopsis and Antirrhinum, came from a homeotic Petunia mutant, called green petals (gpet, now Petunia hybrida DEFICIENS, PhDEF) [33]. In this null mutant, petals fully convert to sepals, but stamen development is unaffected. The reason behind this aberrant phenotype was only discovered by a functional analysis of the Petunia B-function genes that also included the TM6 gene copy (Petunia hybrida TM6, PhTM6) [32,34]. While all aspects of B-regulation described for Arabidopsis and Antirrhinum appear to be conserved for the duplicated pair of Petunia PhGLO genes and for PhDEF (GP), PhTM6 clearly does not obey the ABC rules (Fig. 1). PhTM6 is most highly expressed in whorls three and four, it does not require functional GLO proteins
to maintain high expression levels, and is not involved in petal identity control. Rather, PhTM6 specifies stamen identity in a fully redundant fashion with PhDEF [32]. In fact, all TM6 genes analyzed so far, including representatives from Petunia, tomato, grape, and Gerbera, tend to be expressed at lower levels in the petals, while they are expressed at high levels in stamens and carpels [32,35–37].

The clear difference in function between euAP3 and TM6 genes, at least in Petunia, seems to be largely attributable to a different regulation of the two proteins: a highly conserved and functionally essential 5′ regulatory element present in euAP3 type promoters [38] is completely absent in the PhTM6 5′ regulatory unit. Although PhTM6 is not involved in petal identity control, it can rescue petal development in a phdef mutant background when expressed from a constitutive promoter [32]. It therefore seems that the differences in protein sequence between TM6 and euAP3 genes have not had a major impact on their functional diversification.

Other examples of a different set-up of B-class regulation or function can be found in the monocots, in which two main floral forms can be distinguished.

Animal attracting monocots (e.g. tulips and lilies) have petaloid organs, called tepals, in both the first and second whorls, which have been associated with expansion of the B-gene expression domain to the first floral whorl (Fig. 1) (e.g. [39]). This observation gave rise to the "sliding boundary" hypothesis, which describes how floral diversity can be achieved by outward or inward shifts of B-function gene expression ([40], reviewed in [41]). An analogous "fading borders" model has been proposed to explain gradual transitions in organ morphology in some basal angiosperms (Fig. 1) ([42,43], reviewed in [41]). However, the molecular changes that have allowed modulation of the B-function domain remain to be determined.

In grasses on the other hand, regulation and expression of B-genes in the second and third floral whorls is well conserved [44–47], but in the second floral whorl, where in eudicot flowers petals form, most grasses produce lodicules: small scale-like or fleshy organs that swell at anthesis to open the floret (Fig. 1). Since maize B-function genes are capable of rescuing the corresponding Arabidopsis B-function mutant phenotypes [47], phenomena like these are probably best explained by changes in the target genes of the B-function transcription factors. It will be interesting to try to find out what changes in target genes have occurred and whether changes in the B-function proteins themselves or their interacting partners might have played a role in this.

2.3. The C- and D-function

The Arabidopsis C-function gene AGAMOUS (AG) is involved in the specification of male and female reproductive organ development and in regulating floral meristem determinacy [7,48]. Two additional Arabidopsis AG subfamily genes, SHATTERPROOF1 (SHP1) and SHP2, share largely redundant functions in specifying the fruit dehiscence zone, and function together with AG in carpel development [49,50]. Another closely related Arabidopsis gene is the D-function gene SEEDSTICK (STK). STK is involved in ovule development, and is required for dispersal of the seeds when the fruit matures [50]. In promoting ovule identity, STK acts redundantly with SHP1, SHP2 and AG [50]. The D-function was originally discovered in Petunia [51] and added several years after the ABC-model was originally proposed, to represent genes involved in regulating ovule development. As D-function genes belong to the same MADS-box gene subfamily as C-function genes and several C-function genes were shown to share functions in ovule development with D-function genes, the D-function genes are perhaps better regarded as more specialized C-function genes.

A gene duplication event early in angiosperm evolution led to the divergent C- and D-function gene lineages (AG clade and FLORAL BINDING PROTEIN7/11 (FBP7/11) clade, respectively). Representatives of the D-lineage appear widely conserved across the angiosperms [52]. Thus far, most identified FBP7/11 clade (D-lineage) genes, including core eudicot and grass orthologs, exhibit ovule-specific expression (e.g. [50,51,53–55]). Functional studies in Petunia and rice have shown that the role of D-function genes in the regulation of ovule development is largely conserved between these two species and Arabidopsis (reviewed in [56]).

More recent gene duplications have taken place in the AG clade (C-lineage) both within the grasses [57,58] and the eudicots [52]. These have been followed by functional diversification of the gene copies, resulting in subfunctionalization and probably also neo-functionalization. Comparative analysis of the Arabidopsis and Antirrhinum AG clade genes shows the randomness of subfunctionalization: the genes that are involved in the primary aspects of C-function, PLENA (PLE) and AG, respectively, are actually paralogs [59]. As the divergence of functions between the different AG paralogs in rice and maize is so similar, it is likely that subfunctionalization of these grass AG clade genes has begun before the divergence of these two species [57,58]. Remarkably, in rice, C-function genes might act in conjunction with the YABBY gene DROOPING LEAF (DL) to specify carpel identity [60]. This mechanism seems not conserved in Arabidopsis, as the Arabidopsis DL ortholog, CRABS CLAW (CRC) plays only a partial role in carpel identity [61].

Even the AG subfamily genes of the most basal angiosperms and gymnosperms are expressed in the reproductive tissues, which sug-
gests a deeply conserved role in the production of these tissues (e.g. [42,62,63]). Overall, the C/D-function is probably the most con-
served gene function among the MADS-box genes, even though
many subfunctionalization events and several neofunctionaliza-
tion events have taken place after gene duplications within the AG
subfamily. It is interesting to speculate about the reason for the
high level of conservation for this gene function. It has been sug-
gested before that there might be a constraint on paralogs within a
species such that the sum total of all functions must cover at least
the ancestral function, especially for the AG subfamily, because
of the critical role AG homologs play in reproduction [64]. To fully
uncover the levels of redundancy, and events of subfunctionaliza-
tion and neofunctionalization within the AG subfamily it will be
necessary to functionally analyze the complete set of AG subfam-
ily members from other species, as was done for Arabidopsis [50].
Such an extensive analysis performed on a number of phylogeneti-
cally well chosen species could also shed light on the meaning of
the C/D-lineage split.

2.4. The E-function

The E-function was not included in the original ABC-model, but
added later as it became clear that the A-, B-, and C-function genes
need other co-factors to produce floral organs [65–68]. Floral organ
identity is proposed to be regulated by multimeric complexes of
ABCD proteins (floral quartet model; [69]). In these complexes
the B-, C-, and D-function proteins are thought to be important for
organ-specific gene regulation, while the E-function proteins act
as the mediators for the formation of the protein complexes (e.g.
[70,71]).

The E-function in Arabidopsis is encoded by genes from the
angiosperm-specific SEPALATA (SEP; previously called AGAMOUS-
LIKE2, AGL2) MADS-box gene subfamily [67]. Arabidopsis harbors
four SEP subfamily genes: SEP1–4. The Arabidopsis sep1 sep2 sep3
triple mutant produces sepalid in all floral whorls (hence the sub-
family name SEPALATA) and shows loss of meristem determinacy
in the center of the flower [67]. Addition of the sep4 mutation
resulted in the conversion of all floral organs into leaves [72]. Thus,
only the quadruple mutant exhibits a complete loss of floral organ
identity. The four Arabidopsis SEP genes show a high level of func-
tional redundancy, though the different genes also demonstrate
diverse diversification in functions (e.g. [73]).

Multiple SEP homologs are present in distantly related angiosperm lineages, suggesting that the SEP subfamily has ex-
perienced several early gene duplication events. The two major
lineages, the AGL9 and the AGL2/3/4 clade, are most likely the result
of a pre-angiosperm duplication, as representatives of both clades
are present in the basal angiosperm Amborella [74]. Additional
gene duplications have occurred in eudicots and the grass mono-

As most species have multiple SEP gene copies with often redun-
dant functions, there is only limited functional data available for SEP
genes. So far only two out of the six Petunia SEP genes have been
analyzed in detail. Together with a study in Arabidopsis [73], this
proved that also the D-function requires SEP activity [75]. Despite
a high level of functional redundancy, the Petunia SEP gene copies
do also exhibit diversification in function. Also the two functionally
analyzed Gerbera SEP genes show signs of subfunctionalization:
GERBERA REGULATOR OF CAPITULUM DEVELOPMENTI (GRCDD1) has
a function, specifically in whorl three, while GRCDD2 has a func-
tion, specifically in whorl four [76,77]. The tomato LeMADS-RIN
gene was also shown to have a unique function: the gene seems
involved in the ripening of the tomato fruit [78]. The highly variable
expression patterns of the grass LH1 lineage SEP genes in differ-
ent species suggest variation in their function in specifying organ
identity and determinacy of the spikelet meristem [79]. Functional
diversification of these genes is thought to have played a role in the
diversification of spikelet morphology [80].

In general, the number of SEP genes and their expression pat-
terns vary between species. The contribution of specific SEP genes
to various aspects of flower development differs. Still, all available
data seem to indicate a general function of SEP proteins as medi-
ators of the formation of a set of protein complexes. So far, it has
been impossible to determine if there are conserved functions spec-
cific to SEP gene lineages. Only by obtaining more functional data
we can figure out the exact functions of all SEP genes.

Interestingly, extant gymnosperms do not seem to harbor any
SEP genes. They do however contain the closely related AGAMOUS-
LIKE6 (AGL6) genes (reviewed in [41,81]). Recently, Rijpkema et
al. [82] showed that the Petunia hybridra AGL6 gene (PhAGL6, for-
merly called PETUNIA MADS BOX GENE4, or pMADS4) functions
redundantly with the SEP genes FBP2 and FBP5 in petal and anther
development. Around the same time, the characterization of two
more AGL6 gene mutants was published: both the maize bearded-
ear (bde) gene and the rice Mosaic FLORAL ORGANS1 (MFO1) gene
are involved in the regulation of floral organ identity and floral
meristem determinacy [83,84], and seem to function like SEP genes.
The expression pattern of the Petunia AGL6 gene, and that of its
homologs from other species [82,85,86], further hints at a role in
ovary, ovule and/or gametophyte development, possibly redundant
with other (SEP) MADS-box genes. Conservation of a SEP-like func-
tion for both Petunia, maize and rice AGL6 genes indicates that
comparative SEP functional analyses should also include members
of the AGL6 subfamily. It will be interesting to find out to what
extent AGL6 genes from other species, especially gymnosperms,
perform a similar function.

3. Control and diversification of inflorescence architecture

Angiosperms widely diverged with regard to the moment (i.e.
the season and/or the plant age) that they switch to flowering as
well as to the number and position of flowers that are formed.
Some species generate a single (solitary) flower at the end of a
shoot, while others generate clusters of flowers, known as inflo-
rescences. Inflorescences can be divided into three major classes
based on their mode of development (Fig. 2) [87–89]. In racemes the
shoot apical meristem grows indefinitely (i.e. it is indeterminate).
It generates lateral meristems that terminate by forming a flower,
resulting in a straight axis with many lateral flowers. In cymes, the
apical meristem is determinate and terminates by forming a flower
while growth continues from a lateral (sympodial) meristem that
forms the next “sympodial” inflorescence unit. Panicles occupy an
intermediate position: both apical and lateral meristems initially
continue to grow and generate more lateral meristems and at some
point they all terminate by forming a flower.

Theoretical modeling indicates that inflorescences may have
diverged by alterations in the spatio-temporal regulation of genes
specifying floral or shoot fate of meristems [89]. In a variety of
species, floral meristem identity is specified by widely conserved
transcription factors known as LEAFY (LFY) and APETALAA1 (AP1)
in Arabidopsis, together with the F-box protein UNUSUAL FLO-
RAL ORGANS (UFO). Mutations in LFY and AP1 homologs (partially)
convert flowers into inflorescence shoots in a variety of species
(reviewed in [88]). The importance of UFO was initially underesti-
mated as ufo mutations have at most a very weak floral meristem
identity phenotype and primarily affect the development of petals
and stamens in the flower [90,91]. In contrast, mutations in the
Petunia and tomato UFO-orthologs DOUBLE TOP (DOT) and ANAN-
THA (AN) almost completely block floral identity [92–94]. The weak
ufo phenotype seems to be due to genetic redundancy as expres-
sion of a dominant negative form of UFO in Arabidopsis results in a
strong flower-to-shoot transformation [95].
Although these floral identity genes encode very similar and functionally exchangeable proteins [92, 96], their expression pattern and genetic regulation diverged widely suggesting that the upstream transcriptional circuitry has been extensively rewired during evolution [92]. For example, in Arabidopsis, UFO is expressed in the inflorescence in lateral (floral) meristems, but also on many sites that lack floral identity [97, 98]. Moreover, constitutive expression of UFO or the Petunia ortholog DOT does not alter the timing and positioning of flowers [92, 97]. The limiting factor that determines when and where flowers are formed in Arabidopsis is the transcription of LFY and its immediate target AP1. LFY expression increases during the vegetative phase and when it reaches a certain threshold flowering commences [99–101]. LFY and AP1 expression in the inflorescence is restricted to the lateral floral meristems and is excluded from the apical inflorescence meristem [10, 102]. If, however, LFY or AP1 are constitutively expressed, precocious flowering occurs and the inflorescence apex converts into a solitary flower [103, 104].

Cymes require a more complex regulation of floral fate as both apical and lateral meristems ultimately form flowers, but with a different timing [89]. In cymes like Petunia and tomato, the LFY-homologs ABERRANT LEAF AND FLOWER (ALF) and FALSIFLORA (FA) are expressed in a different and wider pattern than LFY [105, 106]. ALF and FA are expressed during the vegetative phase, while in the inflorescence they are first expressed in apical meristems and with some delay in lateral meristems. The UFO-homologs DOT and AN, however, are expressed in a narrower pattern than UFO, as they are only active during flowering within apical (floral) meristems, while their expression in lateral meristems is delayed, much more than that of ALF [92, 94]. That the transcription of DOT rather than ALF is the factor that delimits the formation of flowers in Petunia is supported by the observation that constitutive expression of DOT or UFO triggers precocious flowering, partially transforms leaves into petals and converts the cyme into a solitary flower – apparently because floral identity is no longer repressed in lateral inflorescence meristems [92].

Recently a new regulator was discovered that seems specific for cymes. EVERGREEN (EVG) from Petunia and COMPOUND INFLORESCENCE (S) of tomato encode a WUSCHEL-RELATED HOMEOBOX (WOX) transcription factor that is required for floral identity. A (near) null evg mutation strongly reduces DOT expression and converts flowers into shoots [107]. Tomato s mutants display a weaker phenotype, possibly because the 3 s alleles – two missense alleles and an unsolved rearrangement – are not null. AN expression in these s mutants is reduced rather than abolished and the formation of flowers is delayed rather than completely inhibited, resulting in increased branching and a more compound inflorescence [94]. Surprisingly, EVG and S are not expressed in the apical floral meristem where DOT is active, but in the newly emerging lateral sympodial meristem shortly before it becomes visible as a separate dome. This together with the finding that mutations like extrapetals and hermit, which convert the cyme into a solitary flower [105, 108], fully repress the floral identity defect of evg, indicates that EVG promotes DOT expression and floral identity indirectly by an unknown mechanism [92].

EVG arose as a paralog of a deeply conserved WOX gene represented by SISTER OF EVERGREEN (SOE) in Petunia and WOX9/STIMPY and WOX8/STIMPY-LIKE in Arabidopsis [107], which are expressed throughout plant development and have important roles in patterning of the embryo and maintenance of a variety of meristems [109–111]. Since Arabidopsis lacks a true EVG homolog with a similar expression pattern and since EVG is fully redundant in Petunia mutants with solitary flowers, it presumably represents a key factor in the evolution of cymose architecture. Given that tomato s mutants phenocopy the more compound cymes of other Solanaceae, it appears that modulation of EVG/S activity was also important for the further diversification of cymes [94].

4. Conclusion

Evo-devo studies on floral development confirm once more the principle of ‘never change a winning team’ in the sense that
the team members largely remain the same. The combinatorial recruitment of MADS-box proteins to specify floral organ identity in angiosperms appears to be cast in iron. The majority of variations on the ABC theme thus far seem to reside in the regulatory circuitry of this winning team, rather than in changes in the protein structure of the respective team members. Better understanding of angiosperm floral diversity at the molecular level therefore might be obtained from an increased focus on the evolution of both cis and trans ABC regulatory elements and variations in downstream target gene control. That being said, it is astonishing to see how in different species sometimes different genes are involved in controlling the same structure (C-function control) and sometimes the same genes induce different structures (LEAFY and UFO in diverse inflorescence types).

Acknowledgement

A.S.R. is funded by Netherlands Organization for Scientific Research grant 825.08.037.

References


